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GENERAL BIOLOGY

Trisomics of Garden Pea (*Pisum sativum* L.) Readily Respond to Selection for Increased Fertility[1]

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Earlier, Berdnikov et al. [1] suggested a hypothesis of the origin of plant B-chromosomes from tertiary trisomics. Tertiary trisomics are organisms with the karyotype supplemented with an exchange chromosome formed from fragments of two chromosomes of the normal set via reciprocal translocation. If the exchange chromosome is small, trisomics may arise in the progeny of heterozygotes for translocation owing to abnormal segregation of a tetravalent in meiosis I. Extraploidy brings about gene imbalance, so, trisomics have a lowered vigor and fertility. However, if the region of the main chromosome set covered by the exchange extrachromosome carries a lethal in a homozygous state, while the extrachromosome carries the normal allele, then only extraploid progeny (trisomics) will be viable. This may happen in a small natural population. Under these conditions, natural selection would favor functional diploidization of trisomics, i.e., accumulation of missense mutations in dose-dependent genes, including those in the extrachromosome. With time, the extrachromosome may become "genetically empty," no longer causing gene imbalance even if present in several doses, and also acquire mechanisms of meiotic drive (preferential transfer to gametes), thereby becoming a B-chromosome.

To test this hypothesis, an experimental model was worked out using Hammarlund's translocation in pea (the first translocation known in the history of genetics), in which one of the exchange chromosomes, 3^6 (composed of fragments of chromosomes 3 and 6), is small [2, 3]. A stable trisomic line Trust-R was obtained, which carried this small exchange chromosome in addition to the normal chromosome complement. To obtain it, the extrachromosome was combined by crossing with the normal karyotype containing the sporophytic lethal *rmv* [1] and the recessive morphological mutation *cri* in a homozygous state. The extrachromosome carried normal alleles of these loci. For an unknown reason, crossing-over between the extrachromosome and the main chromosome set is suppressed in line Trust-R. As a consequence, the normal allele *Rmv* cannot be transferred to an intact chromosome via recombination, making these trisomics true breeding. The obtained true breeding trisomic line underwent isogenization through selfing in the course of 11 generations in each of which one plant being randomly selected for breeding. In this way, a pure line Trust-R was established [1].

Although the trisomics of line Trust-R are robust plants, they still suffer from gene imbalance expressed as retarded growth and lowered fertility. Since any missense mutation in a dose-dependent gene in the extraploid region of the genome would be favorable, we may expect an easy response of line Trust-R to selection for characters dependent on the gene imbalance, first of all, for vigor and fertility of plants. In this respect, trisomics should differ from euploid pure lines, where the response to selection is limited by mutations that change the selected character in the desired direction, which is much less probable.

During the next 11 generations, the line Trust-R was subjected to selection for increased fertility: about 250 plants were sowed in a hydroponic bed in a greenhouse, and seeds from the three most fertile plants contributed to the next generation. The resultant line Trust-RS was propagated, and an experiment was carried out on evaluation of the selection efficiency. In the greenhouse, 114 seeds of line Trust-RS and 114 seeds of the nonselected line Trust-R were sowed in chess-board order. In the resultant plants, a number of quantitative characters were measured. The results are presented in Table 1. Statistically significant differences in many characters were found. First, these were characters related to the rate and duration of growth: the plants of the selected line germinated earlier, flowered somewhat higher on the stem, and had a greater definitive length

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[1] The article was translated by the authors.

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Table 1. Quantitative characters of greenhouse-grown plants of the initial line Trust-R and the line Trust-RS that underwent 11 generations of selection for increased fertility

Character	Trust-RS	Trust-R	Relative effect, %	*T*
Day of appearance of seedlings	8.48 ± 0.16 σ = 1.60 *n* = 103	9.14 ± 0.17 σ = 1.55 *n* = 79	–7.2	–2.80**
Plant height (above node 2) on the 25th day after sowing, mm	403.79 ± 8.92 σ = 89.67 *n* = 101	381.55 ± 9.31 σ = 82.21 *n* = 78	5.8	1.71
Day of flowering	37.91 ± 0.41 σ = 4.11 *n* = 100	38.92 ± 0.67 σ = 5.90 *n* = 77	–2.3	–1.34
Main stem length (above node 2), cm	236.61 ± 2.63 σ = 25.80 *n* = 96	226.61 ± 3.64 σ = 30.25 *n* = 69	4.4	2.28*
Node of flowering	11.88 ± 0.14 σ = 1.36 *n* = 96	11.14 ± 0.15 σ = 1.26 *n* = 71	6.6	3.57***
Number of nodes on the main stem	29.44 ± 0.44 σ = 4.26 *n* = 95	28.62 ± 0.50 σ = 4.18 *n* = 69	2.9	1.23
Number of pedicles without pods on the main stem	14.43 ± 0.47 σ = 4.47 *n* = 92	13.22 ± 0.79 σ = 6.54 *n* = 68	9.2	1.39
Number of pedicles without pods on lateral branches	6.54 ± 0.65 σ = 5.74 *n* = 79	4.92 ± 0.66 σ = 4.74 *n* = 52	32.9	1.70
Number of lateral branches	12.63 ± 0.51 σ = 5.04 *n* = 96	10.17 ± 0.45 σ = 3.76 *n* = 70	24.2	3.44***
Total number of nodes in lateral branches	69.46 ± 3.65 σ = 35.75 *n* = 96	52.20 ± 3.10 σ = 25.96 *n* = 70	33.1	3.43***
Mean number of nodes per lateral branch	5.48 ± 0.15 σ = 1.49 *n* = 96	5.03 ± 0.17 σ = 1.45 *n* = 70	15.3	1.94
Total number of seeds	20.20 ± 1.14 σ = 11.22 *n* = 96	13.40 ± 0.99 σ = 8.27 *n* = 70	50.7	4.29***
Total number of pods	16.04 ± 0.89 σ = 8.73 *n* = 96	12.90 ± 0.90 σ = 7.55 *n* = 70	24.3	2.42*
Total number of pods with seeds	12.32 ± 0.69 σ = 6.75 *n* = 96	8.43 ± 0.61 σ = 5.09 *n* = 70	46.1	4.06***

Table 1. (Contd.)

Character	Trust-RS	Trust-R	Relative effect, %	*T*
Total number of pods without seeds	3.72 ± 0.33 σ = 3.24 *n* = 96	4.47 ± 0.43 σ = 3.63 *n* = 70	–16.8	–1.40
Mean number of seeds per pod	1.30 ± 0.04 σ = 0.40 *n* = 90	1.05 ± 0.05 σ = 0.42 *n* = 66	23.8	3.77***
Mean number of seeds per a non-empty pod	1.67 ± 0.04 σ = 0.33 *n* = 90	1.59 ± 0.05 σ = 0.37 *n* = 65	5.0	1.34
Maximum number of seeds per pod	2.89 ± 0.08 σ = 0.79 *n* = 90	2.72 ± 0.12 σ = 0.98 *n* = 65	6.3	1.19
Mean mass of a seed, mg	204.09 ± 2.18 σ = 20.78 *n* = 91	200.25 ± 4.13 σ = 33.32 *n* = 65	1.9	0.89
Total seed mass, g	4.44 ± 0.23 σ = 2.20 *n* = 90	2.98 ± 0.21 σ = 1.66 *n* = 65	49.0	4.50***
Number of flowers[†], on the main stem	24.82 ± 0.56 σ = 5.35 *n* = 90	21.58 ± 0.87 σ = 6.99 *n* = 65	15.0	3.26***
Total number of flowers	39.40 ± 1.36 σ = 11.98 *n* = 78	33.10 ± 1.74 σ = 12.30 *n* = 50	19.0	2.87**
Mean number of seeds per flower on the main stem	0.52 ± 0.02 σ = 0.20 *n* = 90	0.46 ± 0.03 σ = 0.25 *n* = 65	13.0	1.66
Mean number of seeds per flower in the entire plant	0.57 ± 0.02 σ = 0.19 *n* = 78	0.49 ± 0.03 σ = 0.21 *n* = 50	16.3	2.27

Note: [†] By the number of flowers, the number of pedicles without pods plus the number of pods was meant. Mean ± standard error is shown; σ, standard deviation; *n*, the number of plants analyzed. Significant differences (Student's *t* test): * $P < 5\%$, ** $P < 1\%$, and *** $P < 0.1\%$.

of the main stem. Second, the selection resulted in an increase in the total number of lateral branches and total number of nodes in them. Third, selection appeared to be effective with respect to fertility characters. Plants of the selected line had, on average, more flowers on the main stem, and the whole plant had more pods and pods with seeds (these trisomics form many pods containing no seeds), greater mean numbers of seeds per pod and per flower, greater number of seeds per plant and, hence, their total mass (with the mean seed mass unchanged). The values of relative changes of the characters are rather great, from several percent to 50%. Characters showing greatest changes after selection included the number of lateral branches, which increased by 24.2%; the total number of nodes in them, which increased by 33.1%; the mean number of seeds per pod (increased by 23.8%); and the total number and mass of seeds (increased by 50.7 and 49.0%, respectively). Thus, plants of the selected line showed a strong increase in the general vigor (the effect on the development of lateral branches being stronger compared to the main stem) and fertility.

Since in our experimental system only trisomics are viable, the selected character (fertility) is determined by (1) the frequency of extrachromosome transfer to gametophytes, (2) viability and competing ability of gametophytes bearing the extrachromosome and (3) general vigour of trisomic sporophytes. A question arises as to which of these parameters contributed to the observed increase in fertility. We evaluated the frequency of transmission of the extrachromosome to the next generation in lines Trust-R and Trust-RS. To this end, both lines were crossed in both directions with the tester line Yellow Crispa homozygous for alleles *gp* and *cri* [1]. In crosses with a homozygote for *cri*, the allele *Cri*, which determines the normal phenotype, can be carried only by the extrachromosome. Among the progeny, plants with the normal phenotype *Cri* are trisomics, while plants with the phenotype *cri* are euploids. Hence, we have a system of detection of extrachromosome transmission either maternally or paternally, depending on the direction of the cross. Both the trisomics and the tester line were characterized by a low seed productivity. Nevertheless, we obtained 653 pods containing 565 hybrid seeds. They were sowed and scored for the phenotypes *Cri* and *cri* at the seedling stage (Table 2). The probability of the extrachromosome transmission via the female gametophyte increased after selection from 28 to 33%, however, these differences have a low statistical significance ($P > 0.2$). According to the estimates, its transmission through the male germline remained almost unchanged, although, in this case, we expected some effect since male gametophytes have to compete with each other at the stage of pollen tube growth. We conclude that the observed response to selection for an increased fertility is accounted for by an increase in the vigor of the sporophyte rather than gametophytes, due to a decrease in gene imbalance at the sporophyte stage.

Table 2. Phenotype with respect to *cri* in progenies of reciprocal analyzing crosses of lines Trust-R and Trust-RS with the tester line Yellow Crispa

Cross	Cri	cri	*P* (%)
Trust-R × Yellow Crispa	40	103	28.0
Trust-RS × Yellow Crispa	53	109	32.7
Yellow Crispa × Trust-R	19	104	15.4
Yellow Crispa × Trust-RS	20	115	14.8

Note: *P*, the proportion of plants with phenotype *Cri*, which corresponds to the frequency of transmission of the extrachromosome to the next generation via one of the two germlines.

We have shown that trisomics are characterized by a high sensitivity to directional selection, most probably, due to spontaneous mutations and the variation resource provided by excess copies of genes. Hence, we have modeled one of the stages of B-chromosome origin, according to our hypothesis [1]. This hypothesis implies cross-pollination as a prerequisite. Indeed, B-chromosomes occur in cross-pollinating plant species. The pea is a self-pollinator with a rare spontaneous crossing. It lacks natural B-chromosomes, but the easiness of experimental crossing allowed us to use it as a model object.

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